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Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a biomarker of Acute Kidney Injury (AKI) / Lipocalina associada à gelatinase neutrofílica como biomarcador da Lesão Renal Aguda

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Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a biomarker of Acute Kidney Injury (AKI) / Lipocalina associada à gelatinase neutrofílica como biomarcador da Lesão Renal Aguda

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Professor Doutor Luís Alexandre de Castilho Silva Coentrão

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Abstract

Background: Acute kidney injury (AKI) is a condition associated with a high mortality and morbidity, affecting patients, daily. As a result, it is imperative to identify a suitable diagnostic biomarker, which can identify acute kidney injury, even at its earliest stages, thereby enabling prompt interventions and improving the patients' clinical outcomes. However, because of its limitations, in the last few years, numerous studies have been carried out to identify new biomarkers that enable an earlier detection of acute kidney injury. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is one of the most promising biomarkers, particularly when considering the monitoring of AKI.

Objective: To summarize the biological and physiological data of NGAL and to review clinical studies related to this biomarker.

Methods: A literature review was undertaken focusing on new biomarkers used in the clinical diagnosis of AKI, especially NGAL. To achieve this, a MEDLINE search was undertaken using the terms "acute kidney injury (AKI)", "biomarker" and "Neutrophil gelatinase-associated lipocalin (NGAL)". The selected papers were published between January 2005 and July 2017 and included the above terms in the title and abstract.

Results: 111 papers were analysed, showing that urinary and serum NGAL can predict AKI in several patient population groups (Area under the receiver operator characteristic curve (AUC) mean: 0.81), as well as the clinical outcomes of this condition, such as length of stay, worsening AKI, need for renal replacement therapy and mortality.

Conclusions: NGAL (Neutrophil Gelatinase-Associated Lipocalin) is the most widely studied novel biomarker. It demonstrates a high sensitivity and specificity for the diagnosis of acute kidney injury, as well as some prognostic information.

Keywords: Acute kidney injury; Biomarkers; Neutrophil gelatinase-associated lipocalin.

Resumo

Introdução: A lesão renal aguda é uma condição com elevada morbilidade e mortalidade, que afeta diariamente um grande número de doentes. Deste modo, torna-se imperativo a realização de um diagnóstico precoce, para a instituição de uma intervenção atempada, com vista à obtenção de melhores resultados clínicos. Contudo, dadas as suas limitações, nos últimos anos, vários estudos têm sido realizados no sentido de identificar novos biomarcadores que permitam a deteção precoce desta condição. O Lipocalina associada à gelatinase neutrofílica (NGAL) é um dos biomarcadores mais promissores, especialmente no que se refere à monitorização da lesão renal aguda.
Objetivo: Resumir as características biológicas e fisiológicas do NGAL e fazer uma revisão dos ensaios clínicos relacionados com este biomarcador.
Métodos: Realizou-se uma revisão da literatura, com enfoque nos biomarcadores utilizados para o diagnóstico de lesão renal aguda, especialmente, o NGAL. Com este intuito, efetuou-se uma pesquisa na MEDLINE usando os termos "acute kidney injury (AKI)", "biomarker" e "Neutrophil gelatinase-associated lipocalin (NGAL)". Os artigos publicados entre Janeiro de 2005 e Julho de 2007, cujos termos acima estavam incluídos no título e resumo, foram selecionados.
Resultados: Foram analisados 111 estudos, que mostraram que o NGAL urinário e plasmático conseguem prever a ocorrência de Lesão Renal Aguda em diferentes populações (AUC média: 0.81), bem como resultados clínicos desta condição, como tempo de internamento, agravamento da lesão renal aguda, necessidade de terapia de substituição renal e mortalidade.
Conclusões: O NGAL é um dos biomarcadores mais estudados, cujos resultados demonstraram uma grande sensibilidade e especificidade no diagnóstico da doença, bem como no seu prognóstico.

Palavras-chave: Biomarcadores; Lesão renal aguda; Lipocalina associada à gelatinase neutrofílica.

Introduction

Acute Kidney Injury (AKI) is characterised by the sudden reduction in kidney function which normally occurs within 24-48 hours. This can result in changes in the acid-base balance in addition to fluid and electrolyte levels.[1]

AKI is a common presentation which can affect patients in different medical specialties. It is associated with significant morbidity and mortality[2] including adverse clinical outcomes such as cardiovascular events[3] and chronic kidney disease.[4]

There are various causes of AKI which are generally divided into 3 categories: pre-renal, intrinsic and post-renal causes. Pre-renal causes are the commonest causes of AKI whereby a reduction in the Glomerular Filtration Rate (GFR) results in renal hypoperfusion because of a reduction in effective arterial pressure to the kidney. This is usually caused by some extrinsic event outside of the kidney which subsequently affects the arterial supply to the kidney. In this category, the renal parenchyma is not the primary source for the renal failure but rather, the compensatory mechanisms initiated because of these external pressures, results in AKI. This is important because the damage is potentially reversible. Intrinsic renal causes, however, are the result of specific renal diseases such as acute interstitial nephritis and acute glomerular nephritis. Post-renal causes are generally the result of obstruction affecting the urinary tract causing an obstructive nephropathy.[5]

Unfortunately, for several years there has been no agreement on the precise clinical definition of AKI. This has meant that it is extremely difficult to compare studies or their outcomes.[6]

Subsequently, in 2004, the Acute Dialysis Quality Initiative (ADQI) group established the first criteria for the diagnosis and classification of AKI called the Risk, Injury, Failure, Loss and End-stage Kidney (RIFLE) criteria. These criteria classified AKI into 3 severity groups (risk, injury and failure) according to either changes in serum creatinine (sCr) or urine output (UOP). The criteria also predicted one of 2 outcomes (Loss and End-stage Renal Disease) which was determined by the duration of kidney dysfunction.[6]

The Acute Kidney Injury Network (AKIN) criteria was subsequently developed to improve several weaknesses of the RIFLE criteria. The AKIN criteria classified the injury into three stages and did not require a baseline sCr. Instead, it defined AKI by the increase in sCr of 0.3mg/dL or 1.5 times its initial recorded value.[7] The specific time criteria proved to be a major flaw as some AKI can occur over periods longer than 48 hours.[8]

Most recently, the RIFLE and AKIN criteria were combined to form the Kidney Disease: Improving Global Outcomes (KIDGO) clinical practice guidelines which clinically defined AKI in one of three ways:

1) An increase in sCr of 0.3mg/dL within 48 hours OR
2) An increase in sCr of 1.5 times the base (or its first measurement) OR
3) A UOP <0.5mL/kg/hr for 6 hours.[1]

These most recent clinical guidelines were thought to be advantageous in that they took into account small changes in sCr, unlike the RIFLE criteria, and were not restricted

to a time period of 48 hours, as stipulated in the AKIN criteria.[9] Nevertheless, the use of sCr as a biomarker for AKI also has certain limitations namely that sCr can be affected by other factors unrelated to renal function, the rise in sCr is a delayed response to the initial insult, and sCr provides no information related to the aetiology or the nature of the renal lesion. sCr is therefore purely a marker of function.[10]

There is a consensus however, that it is important to accurately diagnose AKI early, preferably at its cellular stress stage before permanent damage occurs.[11] This will enable the implementation of adequate therapeutics which will improve post-AKI outcomes.[12] Therefore, there is a need to identify sensitive and specific biomarkers which can help to accurately diagnose AKI at the cellular stress stage.[9]

The purpose of this paper is to summarise the biological and physiological data of the most widely studied biomarker Neutrophil Gelatinase-Associated Lipocalin (NGAL) and review the clinical studies related to this biomarker.

Methods

A literature review was undertaken focusing on novel biomarkers used in the clinical diagnosis of AKI, especially NGAL. To achieve this, a MEDLINE search was undertaken between April and August 2017 using the terms “acute kidney injury (AKI)”, “biomarker” and “Neutrophil gelatinase-associated lipocalin (NGAL)”.

Initially, only literature review papers published between January 2013 and July 2017 using the above terms (“acute kidney injury (AKI)” and “biomarker”) included in the title and abstract, were selected. This literature search was subsequently expanded, using the references included in the initially selected literature review papers.

Subsequently, using the term “Neutrophil gelatinase-associated lipocalin (NGAL),” a second search was carried out with the aim of identifying clinical studies which evaluated the capacity for urinary and plasma NGAL to predict AKI in humans. Only publications between January 2005 and July 2017 were used. As described earlier, the title and subsequent review of the abstracts enabled the selection of the most appropriate publications. Only articles which focused on the following patient populations were selected:

- patients undergoing cardiac surgery, with contrast-induced nephropathy (CIN),
- admitted to the Intensive Care Unit (ICU),
- burns,
- after hematopoietic stem cell transplantation (HSCT),
- with drug-induced nephrotoxicity,
- admitted to the Emergency department (ED),
- patients with hypoplastic left heart syndrome (HLHS),
- after liver transplantation and
- patients delay graft function (DGF) following kidney transplantation.

111 clinical trials were selected for critical appraisal with the primary objective of identifying in each case, the following:

- study type,
- sample size,
- population type,
- clinical setting,
- NGAL measurement,
- method of detection,
- AUC,
- AKI definition and
- prognostic outcome.

Retrospectively, the mean and standard deviation of Area under the receiver operator characteristic curve (AUC) were calculated for all studies in addition to each clinical study.

The classic biomarkers of AKI

Renal biomarkers identify whether renal function is normal or impaired.[13] To fulfil this purpose, a good biomarker should have the following characteristics: 1) be easy and simple to measure, 2) be reliable and consistent in repetitive measurements, 3) be cost effective, 4) provide rapid results, 5) be able to identify source of the injury, 6) should differentiate between pre-renal azotemia and chronic kidney disease, 7) enable some estimate of the timing of onset, 8) indicate the severity of injury, 9) enable predictions of patient outcomes (recovery, dialysis and mortality)[14], 10) enable monitoring of pharmacological responses to therapy.[15]

The definition of AKI has traditionally been based on the measure of the sCr level.

Creatinine

Creatinine is a 113 Da protein, formed from creatine in the muscles. In the kidney, it is freely filtered through the glomeruli and is neither absorbed nor metabolized. It is non-toxic and is not bound to any protein. These characteristics make it a practical indicator of GFR.[16]

The proximal tubules are responsible for approximately 10-20% of the excreted load. Therefore, taking this into account, if we use creatinine clearance as a measure of GFR, creatinine clearance will overestimate the GFR. This contribution can be as high as 50% of the creatinine clearance when the glomerular filtration process may actually be reduced.[16]

However, the concentration of sCr is influenced by a lot of factors. There is also a non-linear inverse relationship between sCr and GFR (particularly in patients with near-normal renal function), which means that it may not accurately reflect the degree of renal dysfunction.[5]

Many studies have therefore concluded that sCr is not an adequate marker of renal function. Other limitations of this marker are as follows:

1) Muscle mass is affected by the age, sex, race and weight of the patient. This in turn will influence the production of sCr by the muscle. This variation therefore makes this a poor marker of renal function especially when trying to identify patients with AKI;
2) The renal tubular epithelium also secretes creatinine;
3) Severely ill patients generally are in a hypercatabolic state, which will alter the metabolism of creatinine in AKI;
4) Patients with AKI require aggressive intravenous fluid administration which will likely cause dilution of creatinine;
5) Some drugs may change the tubular secretion of creatinine;
6) Creatinine levels are a late and indirect sign of damage to the kidney;
7) The time frame of urine collection may not be practical or accurate.[5,17]

Serum Creatinine could be considered a kidney function biomarker.[5] However, because of its poor sensitivity and specificity, it is a poor predictor of kidney damage in AKI [18].

The novel biomarkers of AKI

In view of the limitations associated with sCr as a AKI biomarker, in the last few years, there have been a lot of studies looking at a series of other molecules involved in the pathogenesis of AKI with a view to analysing their potential as biomarkers.[19,20]

One of the most investigated biomarker is NGAL is described below.

The characteristics of others promising AKI biomarkers are summarized in table I.

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a small protein (approximately 25 kDa in size), that belongs to the superfamily of the lipocalins. It was originally found in human neutrophils having been localized in their specific granules.[21] However, subsequent studies identified this protein in other immune cells and several tissues and organs (trachea, lung, stomach, liver, colon and kidney).[22]

NGAL exists in several formats including a 45 kDa homodimer which is produced by neutrophils. It also exists as a monomer and a 135 kDa heterodimer which are predominant forms produced by proximal tubular epithelial cells. The heterodimeric form is conjugated to gelatinase.[23]

NGAL has also been found to be produced primarily in the thick ascending loop of Henle and the intercalated cells of the collecting ducts[24]. It is freely filtered through the glomeruli due to its low molecular weight and positive charge. It is subsequently reabsorbed by the proximal tubule, where it is degraded by megalin and partly excreted in urine. Therefore, in healthy subjects, it is expressed in low constant levels, at a concentration of approximately 20 ng/mL, both in the serum and in the urine.[14]

However, NGAL production increases as we get older and is greater in the female population.[25]

Human (neonates, children and adults) and animal model studies shows that NGAL is upregulated and released into the plasma and the urine after an ischemic or toxic injury to the renal tubular cells.[26]

Following cellular damage, NGAL plays a role as a stimulator of epithelial growth by inducing the differentiation of kidney progenitor cells into tubular epithelial cells, thereby enhancing neutrophil apoptosis in renal tubular interstitial cells.[27] This protein has a bacteriostatic function through its ability to sequester iron-siderophore complexes which prevents iron uptake by bacteria.[28]

Following an acute kidney injury event, there is decreased tubular reabsorption of the protein resulting in elevated urinary concentration levels.[19] The levels of NGAL rise three hours after cellular damaged and its concentration peak is detectable approximately 6-12 hours, according to the severity of injury. If the injury is severe, this elevation can persist up to 5 days.[29,30] Subsequently, urine and plasma concentrations increase rapidly and proportionally to the duration and severity of the insult.[31]

Some authors believe that the rise in urine NGAL can differentiate between intrinsic renal injury and hemodynamic alterations (volume depletion).[32,33]

Given the above features of NGAL, it has become the most extensively studied AKI biomarker[20], with some studies looking at variations in several patient population groups (see Table II). It has been studied in the following scenarios as a predictor of AKI in neonates, children and adults undergoing cardiac surgery[29-31,34-65]; children and adults with contrast-induced nephropathy (CIN)[66-77]; critically ill patients admitted to the Intensive Care Unit (ICU)[33,57,78-93]; burn patients[94-98]; patients after hematopoietic stem cell transplantation (HSCT)[99]; patients with drug-induced nephrotoxicity[100-104]; patients admitted to the Emergency department (ED)[32,33,105-111]; patients with hypoplastic left heart syndrome (HLHS)[112]; patients after liver transplantation[113-120] and patients with delay graft function (DGF) following kidney transplantation.[121-141]

111 clinical trials [see Table II] who used NGAL as a predictive marker of AKI were critically appraised. Some trials only evaluated the performance of urinary NGAL (52 clinical trials), or serum NGAL (34 clinical trials), whilst the remainder analysed both serum and urinary NGAL (24 clinical trials) [see Table III]. The majority of the clinical trials were prospective, carried out primarily on patients following cardiac surgery followed by post-renal transplants, ICU admissions and Contrast-Induced Nephropathy (CIN) as illustrated in Figure 1.

We can identify that many of these studies looking at NGAL in different population groups used very small sample sizes. Nevertheless, when comparing different studies looking at the same population groups, similar results were achieved.

Conventional enzyme-linked immunosorbent assay (ELISA) was the principle method used to detect NGAL in these clinical trials in addition to chemiluminescent microparticle immunoassay (CMIA) in the case of urinary NGAL and the Triage Meter in the case of serum NGAL, amongst others.

When analysing the performance of NGAL, we can verify the overall mean AUC from the different studies is 0.810 ± 0.13. In the case of urinary NGAL, the mean AUC is 0.817 ± 0.12 and in plasma NGAL, the mean AUC is 0.800 ± 0.14. However, some of the clinical trials used other statistical methods (such that AUC was not used) to estimate the performance of NGAL. If we compare the mean AUC obtained from the different populations studied [see Table III] we can verify that the results obtained are very similar.

When analysing each different population group, patients who underwent cardiac surgery made up the greatest proportion of patients analysed in these clinical trials with a mean AUC of 0.807 ± 0.14. Over half of the clinical trials only analysed the performance of urinary NGAL. Despite some trials showing some good results, there were some clinical trials which concluded some limited diagnostic accuracy in predicting AKI (AUC ≤ 0.700), regardless of whether urinary or plasma NGAL was analysed in adults.[30,40,61,63] On the other hand, in the trials involving children, results for plasma NGAL were similar to those described in the adult population (AUC < 0.600).[53,65] In one trial looking at the performance of urinary NGAL in adults, an AUC result of 0.500 was obtained which suggests a limited capacity of NGAL to be used as a predictor of AKI.[64] Nevertheless, this was one trial and there are other clinical trials which suggest otherwise.

The measurements of NGAL in patients with delayed graft function (DGF) after a renal transplant was the second most common population group used in the clinical trials selected with a mean AUC of 0.843 ± 0.12, and as with the previous group, this was

made up largely by the analysis of urinary NGAL. In this population group, however there are also contradicting clinical trials. In the adult population, in 3 trials which analysed the performance of both biomarkers, one trial concluded that both biomarkers had a limited capacity to predict AKI.[126] In another, urinary NGAL was not a good predictor of AKI unlike plasma NGAL which was more promising.[134] In the third clinical trial, the opposite conclusion was reached in that the urinary NGAL showed more promising results in the prediction of AKI unlike the plasma NGAL which did not.[133] In the children's population groups, there was one study in which they evaluated both biomarkers which revealed that urinary NGAL is not a good predictor of AKI unlike plasma NGAL.[130] Nevertheless, in studies which analysed both urinary NGAL and plasma NGAL in isolation, both biomarkers demonstrated good results.

In patients admitted to the ICU, the mean AUC was 0.769 ± 0.15. There was a balanced number of trials that looked at urinary and/or plasma NGAL. In the adult population groups, only 3 trials were identified which showed some discrepancies. In 2 of those studies, they concluded that urinary NGAL is a good predictor of AKI unlike plasma NGAL.[80,85] The third trial showed that both urinary and plasma NGAL were poor biomarkers for AKI (AUC < 0.530).[86] However, in the children's population group, one study which looked at plasma NGAL, concluded that plasma NGAL was not a good biomarker for AKI.[88] As previously mentioned, other studies included in this review had differing results and conclusions.

In patients with CIN, the mean AUC was 0.796 ± 0.18 with studies looking at both biomarkers (urinary and plasma) showing some limitation in this role. One study recently published showed that urinary NGAL demonstrates no capacity as a predictor of AKI (AUC 0.510).[71]

In patients who have undergone a liver transplant, the mean AUC was 0.794 ± 0.07 with only one study looking at the performance of plasma NGAL showing some limitation in its diagnostic role in AKI.[120]

In patients who have sustained burns, which represented the smallest number of clinical studies (only 5 identified), the mean AUC was 0.821 ± 0.14, whereby 2 articles which looked at plasma NGAL showed an AUC < 0.700.[96,97]

In the remainder populations, patients admitted to the ED and drug-associated nephrotoxicity, the mean AUC was 0.850 ± 0.08 e 0.847 ± 0.04 respectively, such that all the studies analysed showed some superiority of both urinary and plasma NGAL in the diagnosis of AKI.

Therefore, whilst most of studies show that NGAL is a good predictor of AKI, there are studies which show some discrepancies indicating that NGAL cannot have that function. On the other hand, in clinical studies looking at similar population groups and the performance of urinary and serum NGAL, these studies appear to indicate that one form of NGAL may be better than the other.

In all these studies, AKI was defined using one of the recognised criteria for AKI, namely either RIFLE and/or AKIN. As previously mentioned, these classifications require the use of creatinine. Therefore, despite all the limitations associated with the use of creatinine, these criteria were used as the standard with which to compare the

performance of NGAL as a AKI biomarker. Unfortunately, there is no gold-standard in the diagnosis of AKI.

The NGAL biomarker was also shown to predict the severity and duration of AKI[37,92,105] as well as the clinical outcomes of this condition, such as length of stay[30,35,37,42,50,65], worsening AKI[32,37,92,93,142], renal recovery[143], UCI admission[33], nephrology consultation[33], need for renal replacement therapy (RRT)[32,33,37,65,72,77,81,89,92,93,96,105,122,142] and mortality.[30,32,35,37,42,57,77,81,92,93,96,105,110]

Hasse et al.[144] created "subclinical AKI" as a new category of patients that have risen urinary NGAL levels but a normal serum creatinine level, created "subclinical AKI" as a new category of patients that have risen urinary NGAL levels but a normal serum creatinine level, that is the presence of some renal damage without the loss of renal function simultaneously.

In addition to this group, there exists another group which needs to be recognised, namely those patients in which there is a reduction in renal function in the absence of renal damage, for example pre-renal azotemia. In this case, there are raised levels of creatinine without an increase in the levels of the NGAL biomarker.[145] This indicates that NGAL alone cannot be used to diagnose this conditions in the relevant patients.

Many studies show that both urinary and plasma NGAL are good biomarkers[146,147], however some authors have suggested that the elevation of urinary NGAL concentration is more specific than the elevation of serum concentration.[146] Plasma NGAL may be influenced by various factors, such as bacterial infections, systemic inflammation and malignancy.[148] A venous blood sample is easy to obtain, although it is considered an invasive procedure.[20] Urinary NGAL, however, is relatively unaffected by external factors, although if the patient is anuric, it may not possible to collect a urine sample to enable analysis.[80]

Several clinical studies and reviews have concluded that both plasma and urinary NGAL levels increase much earlier and show better sensitivity than sCr, in the diagnosis and prognosis of AKI.[29,35,146]

Compared with other novel biomarkers, NGAL can diagnose AKI earlier than serum Cystatin C (CysC)[67], but predicts AKI much less effectively than Insulin-like Growth Factor-Binding Protein 7 (IGFBP7) and Tissue Inhibitor of Metalloproteinases-2 (TIMP2).[149] Moreover, the Kidney Injury Molecule 1 (KIM-1) is more specific than NGAL, but it is less sensitive.[20]

To complete this work, it would be important to understand for each population used in the study of NGAL, what the cut-off values would be and the time period which would enable a better prediction of AKI, in addition to its performance when utilised in conjunction with other biomarkers.

Conclusion

In the last few years, there has been much research to identify ways to improve the early diagnosis of AKI. A lot of work has looked into the pathophysiology of AKI to identify several biomarkers in a variety of different population groups with promising results.

NGAL is one of the most promising biomarkers particularly with regards to the monitoring of AKI following several studies in a variety of clinical settings. It is an excellent biomarker with a high sensitivity and specificity as well as the potential to predict a patient's potential clinical outcome (recovery, dialysis and mortality).

However, there is evidence to suggest that it may be influenced by several interference factors and therefore may only be suitable to patient with fewer complications.

In view of this, it may not be possible to use a single biomarker, but rather combine the results of several biomarkers to enhance the diagnostic and prognostic predictions of patients with AKI.

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Appendix

Appendix I – Table I

Biomarker	Characteristics	Mechanism of release	Presence/levels in healthy individuals	Source	Method of detection	AKI predictor	Advantages	Limitations
CysC	Protease inhibitor produced in all nucleated cells. Freely filtered by the glomerulus, completely reabsorbed and degraded but not actively secreted[150]	GFR reduction[14]	Serum levels: yes[151]	Serum[150]	ELISA; nephelometric; Turbidometric assays[14]	Burn patients[152] Adults[35] and children[53,153] patients undergoing cardiac surgery Drug-induced nephrotoxicity[154]	High sensitivity and specificity to GFR[155] In neonates, CysC is independent of maternal values[156,157]	Lack of a standardized test [20] CysC levels may be influenced by thyroid dysfunction, immunosuppressant use, systemic inflammatory response[158] and smoking[155]
NAG	Enzyme produced by lysosomes of the renal proximal tubular cells[159]	Cellular injury[159]	Urinary levels: 15.1 U/L[160]	Urine[159]	ELISA; Spectrophotometric assay[14]	Drug-induced nephrotoxicity[154] Critically ill patients admitted to the ICU[160] CIN[161]	Useful, non-invasive, simple, inexpensive[161]	NAG levels may be influenced by industrial solvents, heavy metals and disease states (rheumatoid arthritis, abnormal glucose tolerance, hyperthyroidism)[20] Urinary elevation may reflect increased lysosomal activity[159]
KIM-1	Transmembrane glycoprotein of the renal proximal tubule[162]	Ischemic or toxic injury[162]	No[162]	Urine[162]/ Serum[163]	ELISA; immunoblotting[14]	CIN[164] Adult patients undergoing cardiac surgery[34,63] Drugs-induced nephrotoxicity[154] Patients admitted to the ED[105]	Non-invasive, not affected by the physicochemical properties of urine[20]	High cost, poor availability[165] KIM-1 levels may be influenced by chronic proteinuria and inflammatory diseases[166]
L-FABP	Cytoplasmic protein of the renal proximal tubule[167]	Ischemic[167] or toxic injury[168]	No[13]	Urine[167]	ELISA[14]	Drug-induced nephrotoxicity[168] Patients in septic shock[169] CIN[170] Adult[171] and children[172] patients undergoing cardiac surgery Critically ill patients admitted to the ICU[173] Patients admitted to the ED[105]	_____	Urinary L-FABP elevation in patients with anaemia[174]

IL-18	Cytokine of the renal proximal tubule[175]	Ischemic or toxic injury[175]	Urinary levels: yes[175]	Urine[175]	ELISA[14]	Patients admitted to the ED[105] DGF following kidney transplantation[121,175] Adults[30] and children[65] patients undergoing cardiac surgery Critically ill patients admitted to the ICU[176]	Detection is fast, reliable and inexpensive[175]	IL-18 levels may be influenced by endotoxin, inflammation and immune factors[177] Serum IL-18 elevation in some pathophysiological conditions (arthritis, SLE, inflammatory bowel disease, psoriasis, hepatitis and multiple sclerosis)[177]
RBP	Protein produced in the liver. Filtered through the glomeruli; reabsorbed and metabolized by the renal proximal tubule [13]	Renal tubular dysfunction[20]	Urinary levels: yes[13]	Urine[13]	ELISA; Nephelometric assay[14]	Drug-induced nephrotoxicity[154,178] Neonates following birth asphyxia[179]	Stable in acid urine[13,20]	Saturation of tubular reabsorption can be limited its detection[20] Heavy glomerular proteinuria or high filtration state may be influenced the RBP specificity as an AKI biomarker[20]
β_2M	Light chain component of the major histocompatibility class I molecule. Filtered through the glomeruli; absorbed and metabolized by the renal proximal tubule[180]	Toxic injury[178]	Serum levels: 2 (0.6 -2.8) mg/L Urinary levels: 80 (3.6 - 320) µg/L[181]	Urine[178]	ELISA; Nephelometric assay[14]	Drug-induced nephrotoxicity[154,178]	_____	Unstable in acid urine[178] Levels β_2M may be influenced by decrease of GFR[178]
IGFBP7 and TIMP2	IGFBP7: secreted protein; TIMP2: protein, apparently, synthesized by renal tubular cells[149]	Sepsis[182] or ischemic injury[183]	Urinary levels: yes[149]	Urine[149]	ELISA; Fluorescence immunoassay[149]	Critically ill adult patients in ICU[149] Adult[184] and children[185] undergoing cardiac surgery	As predictor of AKI, was superior to other biomarkers[149]	Lack studies about their pathophysiology[19]
MicroRNA	Small and noncoding sequences of RNA[186]	Ischemic or toxic injury[186]	Serum and urinary levels: yes[187]	Urine/ Serum[187]	Real-time PCR[186]	CIN[188] Adult undergoing cardiac surgery[187]	High stability, non-invasive, simple, sensitive[186]	Need more clinical studies[189]

Table I. Novel biomarkers of acute kidney injury. Abbreviations: AKI: Acute Kidney Injury; CIN: Contrast-induced nephropathy; CysC: Cystatin C; DGF: Delay graft function; ED: Emergency department; ELISA: Conventional enzyme-linked immunosorbent assay; GFR: Glomerular Filtration Rate; ICU: Intensive Care Unit; IGFBP7: Insulin-like Growth Factor-Binding Protein 7; IL-18: Interleukin-18; KIM-1: Kidney Injury Molecule 1; L-FABP: Liver-type Fatty Acid-Binding protein; NAG: N-Acetyl-β-D-glucosaminidase; PCR: Polymerase Chain Reaction; RBP: Retinol-Binding Protein; RNA: Ribonucleic Acid; SLE: Systemic Lupus Erythematosus; TIMP2: Tissue Inhibitor of Metalloproteinases-2; β_2M: β_2-Microblobulin

Appendix II – Table II

Author	Study type	Sample Size (AKI/ no AKI)	Population type	Clinical setting	NGAL mesurement	Method of detection	AUC for AKI prediction	AKI definition	Prognostic outcome
Mishra et al., 2005[29]	Prospective	71 (20/51)	Children	After cardiac surgery	Urine	ELISA	0.998	≥ 50% increase in sCr concentration from baseline	
					Plasma	ELISA	0.906		
Bachorzewska-Gajewska et al., 2006[68]	Prospective	35	Adults	CIN	Urine	ELISA	Not reported	____	
					Plasma	ELISA	Not reported		
Mishra et al., 2006[124]	Prospective	25	Children	DGF following kidney transplantation	Biopsies	NGAL staining	Not reported	DGF: need for dialysis within the first week after transplantation.	
Parikh et al., 2006[121]	Prospective	53	Adults and children	DGF following kidney transplantation	Urine	ELISA	0.900	DGF: need for dialysis within the first week after transplantation.	
Wagener et al., 2006[39]	Prospective	81 (16/65)	Adults	After cardiac surgery	Urine	ELISA	0.800	≥ 50% increase in sCr concentration compared with preoperative	
Dent et al., 2007[42]	Prospective	120 (45/75)	Children	After cardiac surgery	Plasma	Triage Meter	0.960	RIFLE criteria	Prediction of length of stay and mortality
Hirsch et al., 2007[66]	Prospective	91 (11/80)	Children	CIN	Urine	ELISA	0.920	50% increase in sCr concentration from baseline	
					Plasma	ELISA	0.910		
Zappitelli et al., 2007[78]	Prospective	140	Children	Critically ill patients admitted to the ICU	Urine	ELISA	0.780	Pediatric RIFLE criteria	
Bennett et al., 2008[37]	Prospective	196 (99/97)	Children	After cardiac surgery	Urine	CMIA	0.950	≥ 50% increase in sCr concentration from baseline	Prediction of length of stay, worsening AKI, need for RRT and mortality
Koyner et al., 2008[47]	Prospective	72 (34/38)	Adults	After cardiac surgery	Urine	ELISA	0.710	Increase in sCr ≥ 25% or RRT within first 72h	
Ling et al., 2008[67]	Prospective	150 (13/27)	Adults	CIN	Urine	ELISA	0.734	Increase in sCr ≥ 44.2 µmol/l or ≥ 25 % from baseline 48 – 72h after the procedure	
Nickolas et al., 2008[33]	Prospective	635	Adults	Patients admitted to the ED	Urine	Immunoblot	0.948	RIFLE criteria	Prediction of need for RRT
Vaidya et al., 2008[107]	Prospective	204 (102/102)	Adults	Patients admitted to the ED	Urine	Microbead based assays	0.890	RIFLE criteria	
Wagener et al., 2008[40]	Prospective	426 (85/341)	Adults	After cardiac surgery	Urine	ELISA	0.611	Increase in sCr > 0.3 mg/dL or 50% from baseline 48 h after the surgery	
Wheeler et al., 2008[88]	Prospective	143 (22/121)	Children	Patients with septic shock admitted to the ICU	Plasma	ELISA	0.677	sCr > 2 mg/dL; BUN > 100 mg/dL OR need for RRT	
Xin et al., 2008[52]	Prospective	33 (9/24)	Adults	After cardiac surgery	Urine	ELISA	0.883	Increase in sCr ≥ 50% within 48 h OR UOP < 0.5 mL/kg/hr for > 6h	

Bachorzewska-Gajewska et al., 2009[69]	Prospective	25	Adults	CIN	Urine	ELISA	Not reported	____	
					Plasma	ELISA	Not reported		
Haase-Fielitz et al., 2009[35]	Prospective	100 (23/77)	Adults	After cardiac surgery	Plasma	Triage Meter	0.870	> 50% in creatinine baseline to peak value within the first five postoperative days	Prediction of length of stay and mortality
Han et al., 2009[63]	Prospective	90 (36/54)	Adults	After cardiac surgery	Urine	ELISA	0.650	>0.3 mg/dL or 2-3-fold creatinine rise within first 72h	
Lebkowska et al., 2009[190]	Prospective	41	Adults	DGF following kidney transplantation	Plasma	ELISA	0.990	DGF: need for dialysis within the first week after transplantation.	
Liangos et al., 2009[64]	Prospective	103 (13/90)	Adults	After cardiac surgery	Urine	Immunoassay	0.500	≥50% creatinine rise within first 72h	
Makris et al., 2009[79]	Prospective	31 (11/20)	Adults	Multi-trauma patient admitted to the ICU	Urine	ELISA	0.977	RIFLE criteria	
Niemann et al., 2009[114]	Prospective	59 (27/32)	Adults	After liver transplantation	Plasma	ELISA	0.790	RIFLE criteria	
Siew et al., 2009[83]	Prospective	391 (86/305)	Adults	Critically ill patients admitted to the ICU	Urine	ELISA	0.710	AKIN criteria	
Tuladhar et al., 2009[41]	Prospective	50 (9/41)	Adults	After cardiac surgery	Urine	ELISA	0.960	RIFLE criteria	
					Plasma	ELISA	0.850		
Che et al., 2010 [48]	Retrospective	29 (14/15)	Adults	After cardiac surgery	Urine	ELISA	0.850	RIFLE criteria	
Constantin et al., 2010[84]	Prospective	88 (45/43)	Adults	Critically ill patients admitted to the ICU	Plasma	Triage Meter	0.920	RIFLE criteria	
Cruz et al., 2010[92]	Prospective	301 (133/168)	Adults	Critically ill patients admitted to the ICU	Plasma	Triage Meter	0.780	RIFLE criteria	Prediction of worsening AKI, need for RRT and mortality
Gaspari et al., 2010[102]	Prospective	46 (12/12)	Adults	Cisplatin-associated nephrotoxicity	Urine	ELISA	Not reported	> 25% increase in sCr concentration from baseline	
					Plasma	ELISA	Not reported		
Hall et al., 2010[122]	Prospective	91 (34/57)	Adults	DGF following kidney transplantation	Urine	ELISA	0.780	DGF: need for dialysis within the first week after transplantation.	Prediction of need for RRT
Koyner et al., 2010[34]	Prospective	123 (46/77)	Adults	After cardiac surgery	Urine	ELISA	0.880	AKIN criteria	
Martensson et al., 2010[80]	Prospective	65 (31/34)	Adults	Patients with septic shock admitted to the ICU	Urine	RIA	0.860	AKIN and RIFLE criteria	
					Plasma	RIA	0.670		
Perry et al., 2010[46]	Retrospective	879 (75/804)	Adults	After cardiac surgery	Plasma	Triage Meter	0.754	≥ 50% increase in sCr concentration from baseline	
Portal et al., 2010[117]	Prospective	80 (30/50)	Adults	After liver transplantation	Plasma	ELISA	0.870	AKIN criteria	
Prabhu et al., 2010[56]	Prospective	30 (8/22)	Adults	After cardiac surgery	Plasma	ELISA	0.980	RIFLE criteria	
Shaker et al., 2010[70]	Prospective	30	Adults	CIN	Plasma	ELISA	Not reported		

Shapiro et al., 2010[109]	Prospective	661	Adults	Patients admitted to the ED	Plasma	Triage Meter	0.820	> 0.5 mg /dL increase in sCr OR need for RRT within 72h	
de Geus et al., 2011[81]	Prospective	632 (171/461)	Adults	Critically ill patients admitted to the ICU	Urine	Triage Meter	0.850	RIFLE criteria	Prediction of need for RRT and mortality
					Plasma	Triage Meter	0.800		
de Geus et al., 2011[82]	Prospective	510 (66/444)	Adults	Critically ill patients admitted to the ICU	Urine	Triage Meter	0.800	RIFLE criteria	
					Plasma	Triage Meter	0.760		
Du et al., 2011[106]	Prospective	252	Children	Patients admitted to the ED	Urine	ELISA	0.800	Pediatric RIFLE criteria	
Endre et al., 2011[93]	Prospective	528	Adults	Critically ill patients admitted to the ICU	Urine	ELISA	0.850	AKIN and RIFLE criteria	Prediction of worsening AKI, need for RRT and mortality
Krawczeski et al., 2011[36]	Prospective	38 (8/27)	Neonates	After cardiac surgery	Urine	ELISA	0.950	AKIN criteria	
					Plasma	ELISA	0.950		
Krawczeski et al., 2011[50]	Prospective	220 (60/160)	Children	After cardiac surgery	Urine	ELISA	0.900	≥ 50% in creatinine baseline within the first two postoperative days	Prediction of length of stay.
Heise et al., 2011[49]	Prospective	50	Adults	After cardiac surgery	Urine	ELISA	0.733	AKIN criteria	
Hollmen et al., 2011[125]	Prospective	176	Adults	DGF following kidney transplantation	Urine	CMIA	0.748	DGF: need for dialysis within the first week after transplantation.	
Hollmen et al., 2011[126]	Prospective	275	Adults	DGF following kidney transplantation	Urine	CMIA	0.595	DGF: need for dialysis within the first week after transplantation.	
					Plasma	ELISA	Not reported		
Parikh et al., 2011[30]	Prospective	1219 (60/1159)	Adults	After cardiac surgery	Urine	CMIA	0.670	AKIN and RIFLE criteria	Prediction of length of stay and mortality
					Plasma	Triage Meter	0.700		
Parikh et al., 2011[65]	Prospective	311 (53/258)	Children	After cardiac surgery	Urine	CMIA	0.710	AKIN and RIFLE criteria	Prediction of length of stay and need for RRT
					Plasma	Triage Meter	0.560		
Singer et al., 2011[32]	Prospective	145	Adults	Patients admitted to the ED	Urine	CMIA	0.870	RIFLE criteria	Prediction of worsening AKI, need for RRT and mortality
Wagener et al., 2011[119]	Prospective	92	Adults	After liver transplantation	Urine	ELISA	0.800	RIFLE criteria	
Cheng et al., 2012[115]	Prospective	26 (13/13)	Adults	After liver transplantation	Plasma	ELISA	0.781	AKIN criteria	
Fadel et al., 2012[43]	Prospective	40	Children	After cardiac surgery	Plasma	ELISA	Not reported	Pediatric RIFLE criteria	
Heyne et al., 2012[128]	Prospective	182	Adults	DGF following kidney transplantation	Urine	ELISA	0.980	DGF: need for dialysis within the first week after transplantation	
Lee et al.,2012[129]	Retrospective	59	Adults	DGF following kidney transplantation	Plasma	ELISA	0.860	DGF: need for dialysis within the first week after transplantation	
Li et al., 2012[113]	Prospective	25 (11/14)	Adults	After liver transplantation	Urine	ELISA	0.773	AKIN criteria	

Magnusson et al., 2012[127]	Prospective	111	Adults	DGF following kidney transplantation	Plasma	ELISA	Not reported	DGF: need for dialysis within the first week after transplantation	
Nickolas et al., 2012[105]	Prospective	1635	Adults	Patients admitted to the ED	Urine	CMIA	0.810	RIFLE criteria	Prediction of need for RRT and mortality
Rahimzadeh et al., 2012[130]	Prospective	27	Children	DGF following kidney transplantation	Urine	ELISA	Not reported	DGF: need for dialysis within the first week after transplantation	
					Plasma	ELISA	0.950		
Royakkers et al., 2012[86]	Prospective	140	Adults	Critically ill patients admitted to the ICU	Urine	ELISA	0.480	RIFLE criteria	
					Plasma	ELISA	0.530		
Salamzadeh et al., 2012[139]	Prospective	68 (11/57)	Adults	DGF following kidney transplantation	Urine	ELISA	Not reported	DGF: need for dialysis within the first week after transplantation	
Aydogdu et al., 2013[85]	Prospective	151	Adults	Patients with sepsis admitted to the ICU	Urine	ELISA	0.800	RIFLE criteria	
					Plasma	ELISA	0.440		
Cho et al., 2013[87]	Prospective	145 (54/91)	Adults	Critically ill patients admitted to the ICU	Urine	ELISA	0.773	AKIN criteria	
Choi et al., 2013[135]	Prospective	69	Adults	DGF following kidney transplantation	Urine	ELISA	0.778	DGF: need for dialysis within the first week after transplantation	
Dedeoglu et al., 2013[116]	Prospective	26 (9/17)	Adults	After liver transplantation	Urine	Triage Meter	0.800	AKIN criteria	
					Plasma	Triage Meter	0.860		
Fonseca et al., 2013[136]	Prospective	40 (18/22)	Adults	DGF following kidney transplantation	Urine	CMIA	0.990	DGF: need for dialysis within the first week after transplantation	
Hong et al., 2013[95]	Prospective	45 (11/34)	Adults	Burned	Plasma	Triage Meter	0.903	RIFLE criteria	
Khosravi et al., 2013[120]	Prospective	90 (31/59)	Adults	After liver transplantation	Plasma	ELISA	0.640	≥ 0.3 mg/dL in creatinine baseline within the first two postoperative days	
Liebetrau et al., 2013[54]	Prospective	141 (47/94)	Adults	After cardiac surgery	Urine	CMIA	0.900	KDIGO criteria	
Lin et al., 2013[104]	Prospective	33 (10/23)	Adults	Cisplatin-associated nephrotoxicity	Urine	ELISA	0.865	____	
Liu et al., 2013[55]	Prospective	109 (26/83)	Adults	After cardiac surgery	Urine	ELISA	0.871	AKIN criteria	
Peco-Antic et al., 2013[53]	Prospective	112 (18/94)	Children	After cardiac surgery	Urine	ELISA	0.930	> 25% decrease in estimated creatinine clearance at 48h after surgery	
					Plasma	ELISA	0.450		
Pickering et al., 2013[89]	Prospective	528	Adults	Critically ill patients admitted to the ICU	Plasma	Triage Meter	0.790	RIFLE criteria	Prediction of need for RRT
Rostami et al., 2013[131]	Prospective	64	Adults	DGF following kidney transplantation	Urine	ELISA	0.713	DGF: need for dialysis within the first week after transplantation	

Schinstock et al., 2013[108]	Prospective	363	Adults	Patients admitted to the ED	Urine	ELISA	0.700	AKIN criteria	
Sirota et al., 2013[118]	Prospective	40 (7/33)	Adults	After liver transplantation	Urine	ELISA	0.833	RIFLE and AKIN criteria	
Soto et al., 2013[111]	Prospective	616	Adults	Patients admitted to the ED	Plasma	Triage Meter	0.890	AKIN and RIFLE criteria	
Valette et al., 2013[72]	Prospective	98 (30/68)	Adults	CIN	Plasma	Triage Meter	0.610	AKIN criteria	Prediction of need for RRT
Zheng et al., 2013[59]	Prospective	58 (29/29)	Children	After cardiac surgery	Urine	ELISA	0.859	AKIN criteria	
Alcaraz et al., 2014[57]	Prospective	106 (36/70)	Children	After cardiac surgery	Urine	CMIA	0.860	Pediatric RIFLE criteria	
Buemi et al., 2014[134]	Prospective	97	Adults	DGF following kidney transplantation	Urine	Sandwich enzyme immunoassay and Triage Meter	Not reported	DGF: need for dialysis within the first week after transplantation	
					Plasma	Sandwich enzyme immunoassay and Triage Meter	0.850		
Hollmen et al., 2014[141]	Prospective	176	Adults	DGF following kidney transplantation	Plasma	ELISA and Triage Meter	0.853	DGF: need for dialysis within the first week after transplantation	
Kidher et al., 2014[44]	Prospective	53 (16/37)	Adults	After cardiac surgery	Plasma	Triage Meter	0.830	RIFLE criteria	
Kim et al., 2014[45]	Retrospective	37 (6/31)	Adults	After cardiac surgery	Plasma	Triage Meter	Not reported	AKIN criteria	
Liebetrau et al., 2014[73]	Prospective	128 (14/114)	Adults	CIN	Urine	CMIA	0.939	KDIGO criteria	
Padhy et al., 2014[74]	Prospective	250 (30/220)	Adults	CIN	Plasma	ELISA	1.000	≥ 0.5 mg/dL in creatinine baseline within the first 48h	
Pajek et al., 2014[138]	Prospective	71	Adults	DGF following kidney transplantation	Urine	ELISA	0.820	DGF: need for dialysis within the first week after transplantation	
Qurashi et al., 2014[140]	Prospective	67	Adults	DGF following kidney transplantation	Urine	CMIA	Not reported	DGF: need for dialysis within the first week after transplantation	
Tsuchimoto et al., 2014[103]	Prospective	93	Adults	Tacrolimus-associated nephrotoxicity	Urine	ELISA	0.876	AKIN criteria	
Yavuz et al., 2014[98]	Prospective	22 (6/16)	Children	Burned	Urine	ELISA	0.960	RIFLE criteria	
					Plasma	ELISA	0.940		
Zeng et al., 2014[58]	Prospective	197 (37/160)	Adults	After cardiac surgery	Urine	ELISA	0.830	AKIN criteria	

Cantaluppi et al., 2015[132]	Prospective	50 (14/36)	Adults	DGF following kidney transplantation	Plasma	Triage Meter	0.940	DGF: need for dialysis within the first week after transplantation	
Lichosik et al., 2015[76]	Prospective	33	Children	CIN	Urine	ELISA	0.998	Pediatric RIFLE criteria	
					Plasma	ELISA	0.910		
Pianta et al., 2015[137]	Prospective	81	Adults	DGF following kidney transplantation	Urine	ELISA	0.770	DGF: need for dialysis within the first week after transplantation	
Quintavalle et al., 2015[77]	Prospective	458 (64/394)	Adults	CIN	Urine	CMIA	0.610	≥ 0.3 mg/dL in creatinine baseline within the first 48h	Prediction of need for RRT and mortality
					Plasma	ELISA	0.620		
Seker et al., 2015[100]	Prospective	42	Adults	Platin-associated nephrotoxicity	Urine	CMIA	Not reported	____	
Sen et al., 2015[94]	Prospective	30 (14/16)	Adults	Burned	Plasma	Triage Meter	0.820	RIFLE criteria	
Shahbazi et al., 2015[101]	Prospective	24 (2/22)	Adults	Cisplatin-associated nephrotoxicity	Urine	____	0.800	AKIN criteria	
Surmiak et al., 2015[112]	Prospective	21 (8/13)	Neonates	HLHS	Plasma	Sandwich enzyme immunoassay	0.920	AKIN criteria	
Taghizadeh-Ghehi et al., 2015[99]	Prospective	72 (12/60)	Adults	After HSCT	Urine	ELISA	0.860	RIFLE and AKIN criteria	
Benzer et al., 2016[75]	Prospective	141 (91/50)	Children	CIN	Plasma	Sandwich enzyme immunoassay	Not reported	Pediatric RIFLE criteria	
Fanning et al., 2016[51]	Prospective	50 (31/19)	Adults	After cardiac surgery	Urine	ELISA	0.712	KDIGO criteria	
Huang et al., 2016[90]	Prospective	76 (30/46)	Adults	Patients with sepsis admitted to the ICU	Plasma	ELISA	0.731	RIFLE criteria	
Kamis et al., 2016[91]	Prospective	107 (38/69)	Adults	Critically ill patients admitted to the ICU	Urine	ELISA	0.930	RIFLE criteria	
					Plasma	ELISA	1.000		
Lacquaniti et al., 2016[133]	Prospective	29 (22/7)	Adults	DGF following kidney transplantation	Urine	ELISA	0.979	DGF: need for dialysis within the first week after transplantation	
					Plasma	ELISA	0.670		
Moriyama et al., 2016[62]	Prospective	50 (11/39)	Adults	After cardiac surgery	Urine	ELISA	0.869	KDIGO criteria	
Rakkolainen et al., 2016[97]	Prospective	19 (9/10)	Adults	Burned	Plasma	Triage Meter	0.623	AKIN criteria	
Chun et al., 2017[96]	Prospective	76	Adults	Burned	Plasma	Triage Meter	0.679	AKIN criteria	Prediction of need for RRT and mortality
Dong et al., 2017[60]	Prospective	150 (50/100)	Children	After cardiac surgery	Urine	ELISA	>0.900	KDIGO criteria	
Hang et al., 2017[110]	Prospective	249	Adults	Patients admitted to the ED	Plasma	Triage Meter	0.923	Increase in sCr ≥ 0.3mg/dL (26.5 mmol/L) within 48 h; increase 1.5 times in creatinine baseline within the prior 7 days OR UOP < 0.5 ml/kg/h for 6 h.	Prediction of mortality.

Kalisnik et al., 2017[61]	Prospective	41 (18/23)	Adults	After cardiac surgery	Plasma	ELISA	0.669	≥ 150% in creatinine baseline OR > 26.4 mmol/L within the first two postoperative days	
Ribitsch et al., 2017[71]	Prospective	617 (58/559)	Adults	CIN	Urine	CMIA	0.510	RIFLE criteria	

Table II. Performance of Neutrophil Gelatinase-Associated Lipocalin in clinical studies. Abbreviations: AKIN: Acute Kidney Injury Network criteria; AUC: area under the receiver operator characteristic curve; BUN: blood urea nitrogen; CIN: Contrast-induced nephropathy; CMIA: chemiluminescent microparticle immunoassay; DGF: Delay graft function; ED: Emergency department; ELISA: Conventional enzyme-linked immunosorbent assay; HLHS: hypoplastic left heart syndrome; HSCT: hematopoietic stem cell transplantation; ICU: intensive care unit; NGAL: Neutrophil Gelatinase-Associated Lipocalin; RIA: polyclonal antibody based radioimmunoassay; RIFLE: Risk, Injury, Failure, Loss and End-stage Kidney criteria; RRT: renal replacement therapy; sCr: serum creatinine; UOP: urine output.

Appendix III – Figure 1

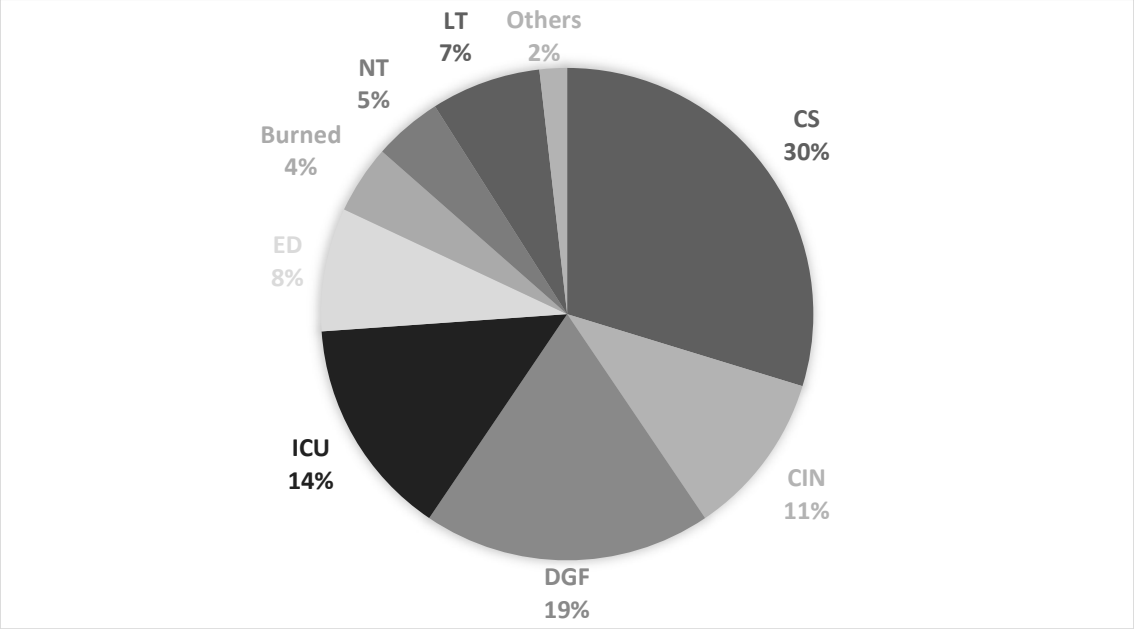

Figure 1. Clinical setting. Abbreviations: CIN: Contrast-induced nephropathy; CS: After cardiac surgery**;** DGF: Delay graft function following kidney transplantation; ED: patients admitted to the Emergency department; ICU: patients admitted to the intensive care unit; LT: After liver transplantation; NT: drug-associated nephrotoxicity.

Appendix IV – Table III

Clinical setting	Clinical studies number			AUC		
	Urine	Plasma	Urine / Plasma	Urine *Mean (SD)*	Plasma *Mean (SD)*	Total *Mean (SD)*
After cardiac surgery	19	8	6	0.816 (0.13)	0.790 (0.17)	0.807 (0.14)
DGF following kidney transplantation	11	5	4	0.823 (0.13)	0.873 (0.11)	0.843 (0.12)
Patients admitted to the ICU	5	5	6	0.800 (0.13)	0.736 (0.16)	0.769 (0.15)
CIN	3	4	5	0.785 (0.20)	0.810 (0.18)	0.797 (0.18)
Patients admitted to the ED	6	3	0	0.836 (0.09)	0.878 (0.05)	0.850 (0.08)
After liver transplantation	3	4	1	0.802 (0.02)	0.788 (0.09)	0.794 (0.07)
Drug-associated nephrotoxicity	4	0	1	0.847 (0.04)	-	0.847 (0.04)
Burned	0	4	1	-	0.793 (0.14)	0.821 (0.14)

Table III. Clinical studies analyses. Abbreviations: AUC: area under the receiver operator characteristic curve; CIN: Contrast-induced nephropathy; DGF: Delay graft function; ED: Emergency department; ICU: intensive care unit; SD: Standard Deviation.

Appendix V - Portuguese Journal of Nephrology and Hypertension: Instructions to Authors

AIMS AND SCOPE

The *Portuguese Journal of Nephrology and Hypertension* is the official organ of the Portuguese Society of Nephrology and is published quarterly. Supplementary issues are also published including selected themes, at the discretion of the Editor-in-Chief, as well as abstracts of the annual congresses of the Society. The Journal is peer-reviewed and is indexed in Thompson Reuter's SciELO Citation Index, with free online access in our website http://www.spnefro.pt/RPNH.

The Journal publishes articles on clinical or laboratory topics of relevance to nephrology, dialysis, transplantation and hypertension. Papers relating to basic immunology, physiology, genetics and epidemiology are accepted when kidney-related. Manuscripts must be submitted in English to the Editor-in-Chief. Only previously unpublished work should be submitted. The Editor-in-Chief has complete editorial freedom.

The Journal complies with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the ICMJE (International Committee of Medical Journal Editors).

Visit http://www.icmje.org

REVIEW AND PUBLICATION SPEED

All submissions will be subject to an immediate editorial screening process by the Editor-in-Chief after which they will normally be sent to two or three reviewers. The Editor-in-Chief will make every effort to reach a decision on all submitted papers within 8 weeks of receipt. Papers will normally be published in the next issue to go to press after their acceptance. Papers that do not meet the scientific standards of the Journal may be declined by the Editor-in-Chief without further review.

CONTENT TYPES

The Portuguese Journal of Nephrology and Hypertension publishes: 1) Editorials; 2) Review Articles; 3) Original Articles; 4) Case Reports; 5) Letters to the Editor; 6) Nephropathology Quiz; 7) Perspective; 8) Comments.

Editorials

Editorials are usually invited, but authors may propose a paper for the Editor-in-Chief's consideration. They may have up to 2000 words and a maximum of 2 tables or figures. A maximum of 5 references is generally recommended.

Review Articles

Review articles should provide novel insights and comprehensive analyses of topics on Nephrology, and interpretation of the published literature. They are usually commissioned by the Editors. However, unsolicited reviews will be considered. These articles may have up to 5000 words and an abstract of up to 300 words. The use of 3 tables or figures is acceptable. A maximum of 70 references is generally recommended.

Original Articles

An original article must focus on relevant clinical investigation or basic research, and is limited to 4000 words including an abstract with up to 300 words. The order of the text should be as follows: Introduction, Subjects and Methods (any statistical method must be detailed in this section), Results and Discussion. A maximum of 50 references is generally recommended.

Case Reports

Original and succinct description structured in Introduction, Case Report and Discussion. They should not exceed 2500 words (including an abstract up to 300 words) and should not include more than 4 tables or figures. A maximum of 30 references is generally recommended.

Letters to the Editor

Letters must contain information related to an article published in the Journal or may concern a topic of current interest in Nephrology. Letters (maximum of 3 authors) are limited to 500 words and 1 table or figure. A maximum of 5 references is generally recommended.

Nephropathology Quiz

A case report to educate clinicians on the renal pathology. This section includes a concise clinical history, images of histology and discussion. These articles are usually invited and are limited to 2000 words, 8 figures and 20 references.

Perspective

Perspective articles are brief, accessible pieces covering a wide variety of timely topics of relevance to health care and medicine. They are nearly always solicited, although unsolicited articles may occasionally be considered. Perspective articles are limited to 1000 to 1200 words and may include one figure or table. A maximum of 8 references is generally recommended.

Comments

Comments usually provide commentary and analysis concerning an article in the issue of the Journal in which they appear. They may also provide commentary concerning an article published elsewhere. They may include 1 figure or table. They are nearly always solicited, although unsolicited comments may occasionally be considered. Comments are usually limited to 1000 words, with up to 10 references.

INSTRUCTIONS FOR AUTHORS

Manuscripts must be submitted online http://rpnh.spnefro.pt. Once you have prepared your manuscript according to the Instructions below, please pay particular attention to the sections on Informed Consent and Ethics and Disclosure.

The text should be double-spaced. The corresponding author should describe the contributions of all authors to the article. Manuscripts should bear the name, address and e-mail of the corresponding author.

Should the manuscript be accepted for publication the authors will be asked to give signed consent for publication in a letter which must contain the statement that "the results presented in this paper have not been published previously, in whole or in part, except in abstract form".

Title Page: The title page should carry the full title of the paper and the first name, middle initial (if applicable) and last name of each author, plus the names and addresses of the respective institutions where the work was done; in the case of different institutions the author(s) should be identified using superscript Arabic numerals.

Abstract: Not more than 300 words. Abbreviations should not be used.

Key-Words: Not more than 6, in alphabetical order, and the terms used (when possible) should be from the Medical Subject Headings list of the Index Medicus.

References: Authors are responsible for bibliographic accuracy. All the references, including those with only electronic sources, should be cited according to the "Vancouver Citation Style" which can be consulted on the Internet at: http://library.vcc.ca/downloads/VCC_VancouverStyleGuide.pdf

References must be numbered consecutively in the order in which they are cited in the text. Each reference should give the name and initials of all authors unless they are more than six, when only the first three should be given followed by et al. Authors' names should be followed by the title of the article, journal abbreviations according to the style used in Index Medicus, the year of publication, the volume number and the first and last page numbers. For papers in the course of publication, "in press" replaces the date; the journal name must be given in the references. Manuscripts that are unpublished, in preparation, or submitted, and personal communications should not be cited in the reference list but may appear parenthetically in the text. References to books should contain the author(s) name(s) and initials, the title of the book, followed by place of publication, publisher, year, and relevant pages. Websites must be referenced by the following order: title, URL and access date.

Examples

1. *Journals*:
Hogan J, Mohan P, Appel GB. Diagnostic tests and treatment options in glomerular disease: 2014 update. Am J Kidney Dis 2014;63(4):656-666

2. *Books*:
Morris Peter, Knechtle Stuart. Kidney Transplantation - Principles and Practice. 7th Edition. Saunders, 2014:72

3. *Website*:
Substitutive Renal Therapy of Chronic Renal Disease in Portugal. Available at http:www.spnefro.pt/comissoes_Gabinete_registo_2013/registo_2013. Accessed October 6, 2013.

4. *Published Meeting Abstract*:
Jorge Silva, Jorge Antunes, Telmo Carvalho, Pedro Ponce. Efficacy of preventing hemodialysis catheter infections with citrate lock (Encontro Renal abstract SE001). Port J Nephrol Hypert 2011; 25(1):56

Tables: Tables should supplement, not duplicate, the information in the main text. References to tables should be made in order of appearance in the text and should be in Roman numerals in brackets, e.g. (Table II). Each table should be typed on a separate sheet and have a brief heading describing its contents.

Figures: All illustrations (transparencies, photographs, diagrams, graphs, etc.) should be labelled consecutively in Arabic numerals (Fig. 1, 2...), according to their relative positions in the text. If a figure has been published before, the original source must be acknowledged and written permission from the copyright holder must be submitted with the material.

Informed Consent and Ethics: Identifying details of patients should not be published in descriptions unless the information is essential for scientific purposes and the patient gives written informed consent for publication. Patients shown in photographs should have their identity obscured or the picture must be accompanied by written permission to use the photograph.

When reporting experiments on human subjects, it is mandatory to indicate whether the procedures were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975 (revised in 2015) and, in the case of renal transplant, the Declaration of Istanbul.

When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Disclosure: Each manuscript must include a conflict of interest statement before the References section. The disclosure statement will describe the sources of any support for the work in the form of grants, consulting fees or honoraria from industry, equipment, provision of drugs, travel related with the study or any combination thereof. Any relevant financial activities outside the submitted paper but considered stakeholders in the field must be detailed. The corresponding author should provide a Conflict of Interest Declaration describing the possible financial interests of all the authors. The absence of any interest must also be declared.

Acknowledgements should be located in the manuscript body before the conflict of interest statement.